IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellants: Stephanie Aquin, Oliver P. Peoples, and Kristi D. Snell

Serial No.: 09/991,152 Art Unit: 1638

Filed: November 16, 2001 Examiner: McElwain, Elizabeth F.

For: *PRODUCTION OF MEDIUM CHAIN LENGTH POLYHYDROXYALKANOATES FROM FATTY ACID BIOSYNTHESIS*

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REPLY UNDER 37 C.F.R. 1.111

Sir:

Responsive to the Examiner's Answer and Advisory Action mailed on October 18, 2007, citing entirely new prior art against the pending claims, which clearly constitutes a new ground of rejection in the above-referenced application, please consider the following remarks. This is an appeal from the final rejection of claims 1, 3-13, 15-20, 22-26 and 29-30 in the Office Action mailed May 26, 2006, and the new grounds of rejections raised in the Examiner's Answer mailed in October 18, 2007, in the above-identified patent application. Please note a Request for Oral Hearing was filed with the Reply Brief on July 2, 2007, and the appropriate fees paid.

It is believed that no additional fee is required with this submission. However, should a fee be required, the Commissioner is hereby authorized to charge the fee to Deposit Account No. 50-3129.

I. The Examiner's Answer Improperly Cites New Prior Art and Prosecution Should have been Reopened.

The following section from the MPEP provides in relevant part (emphasis added):

1207.03 < New Ground of Rejection in Examiner's Answer [R-3]

37 CFR **>41.39(a)(2) permits< the entry of a new ground of rejection in an examiner's answer mailed on or after September 13, 2004. New grounds of rejection in an examiner's answer are envisioned to be rare, rather than a routine occurrence. For example, where appellant made a new argument for the first time in the appeal brief, the examiner may include a new ground of rejection in an examiner's answer to address the newly presented argument **by adding a secondary reference from the prior art on the record**. New grounds of rejection are not limited to only a rejection made in response to an argument presented for the first time in an appeal brief. At the time of preparing the answer to an appeal brief, the examiner may decide that he or she should apply a new ground of rejection against some or all of the appealed claims. In such an instance where a new ground of rejection is necessary, the examiner should either reopen prosecution or set forth the new ground of rejection in the answer. The examiner must obtain supervisory approval in order to reopen prosecution after an appeal. See MPEP § **1002.02**(d) and § **1207.04**.

I. REQUIREMENTS FOR A NEW GROUND OF REJECTION

Any new ground of rejection made by an examiner in an answer must be:

(A) approved by a Technology Center (TC) Director or designee; and

(B) prominently identified in the "Grounds of Rejection to be Reviewed on Appeal" section and the "Grounds of Rejection" section of the answer (see MPEP § **1207.02**). The examiner may use form paragraph 12.154.04.

The examiner's answer must provide appellant a two-month time period for reply. The examiner may use form paragraph 12.179.01 to notify appellant of the period for reply and to include the approval of the TC Director or designee. In response to an examiner's answer that contains a new ground of rejection, appellant must either file: (A) a reply in compliance with 37 CFR **1.111** to request that prosecution be reopened; or (B) a reply brief that addresses each new ground of rejection in compliance with 37 CFR **41.37**(c)(1)(vii) to maintain the appeal.

II. SITUATIONS WHERE NEW GROUNDS OF REJECTION ARE NOT PERMISSIBLE

A new ground of rejection would not be permitted to reject a previously allowed or objected to claim even if the new ground of rejection would rely upon evidence already of record. In this instance, rather than making a new ground of rejection in an examiner's answer, if the basis for the new ground of rejection was approved by a supervisory patent examiner as currently set forth in MPEP § **1207.04**, the examiner would reopen prosecution. In addition, if an appellant has clearly set forth an argument in a previous reply during prosecution of the application and the examiner has failed to address that argument, the examiner would not be permitted to add a new ground of rejection in the examiner's answer to respond to that argument but would be permitted to reopen prosecution, if appropriate. New grounds of rejection cannot be made in a

supplemental examiner's answer unless it is written in response to a remand by the Board for further consideration of a rejection under 37 CFR **41.50**(a).

It is very clear from the foregoing that the examiner has committed multiple errors in the Examiner's Answer:

(1) Cite new prior art against the claims under 35 U.S.C. 102 or 103, as a primary reference.

(2) Failed to identify the new rejection as a new ground of rejection.

(3) Failed to obtain approval to make the new ground of rejection or reopen prosecution.

Accordingly, prosecution should either be reopened or the grounds of rejection withdrawn.

II. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Based on the Examiner's Answer, the issues present on appeal are:

(i) whether claims 1, 3-9, 11 and 13-26 are anticipated under 35 U.S.C. §102(a) by Poirier, et al., *Plant Physiol.*, 121:1359-66 (1999) ("Poirier"). **This is a new ground of rejection.**

(ii) whether claims 1, 3-13, 15-20, 22-26, 29 and 30 are obvious under 35 U.S.C. §103(a) over Poirier, in view of U.S. Patent No. 5,750,848 to Kruger, et al., ("Kruger"). **This is a new ground of rejection.**

(iii) whether claims 1, 3-13, 15-20, 22-26, 29 and 30 are obvious under 35 U.S.C. § 103(a) in view of U.S. Patent No. 5,750,848 to Kruger, et al., ("Kruger").

III. ARGUMENTS

(a) The Claimed Invention

Neither of the cited references either anticipate or make obvious the claimed subject matter. This is because the claims and each of the references utilize different metabolic

pathways. Due to the complexity of the metabolic pathways involved in the specification and the two cited references, applicants feel that an explanation of these pathways may be helpful.

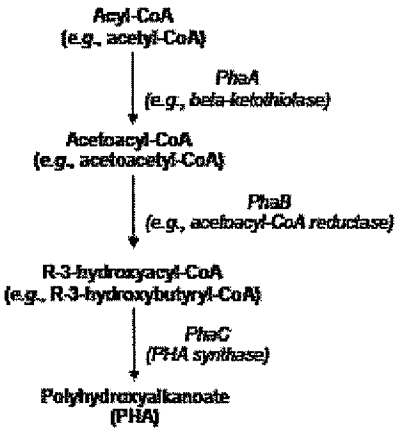
The Metabolic Pathways

The enzymes in the polyhydroxyalkanoate (PHA) biosynthetic pathway occurring naturally in *R. eutropha* and Pseudomonads are described on page 1, line 22 to page 2, line 4 of the present application. Specifically, the native pathway in *R. eutropha* typically involves:

- the conversion of acyl-CoA to acetoacyl-CoA by the action of a beta-ketothiolase enzyme (such as the enzyme encoded by the gene *phaA*), then
- the conversion of acetoacyl-CoA to a R-3-hydroxyacyl-CoA by the action of an acetoacyl-CoA reductase (such as the enzyme encoded by the gene *phaB*), then
- production of the PHA polymer utilizing a PHA synthase enzyme (such as the enzyme encoded by the gene *phaC*), which catalyzes the polymerization of the intermediate R-3-hydroxyacid-CoA to form PHA.

This is shown as follows:

(Diagram A)



In this native pathway, the precise monomer composition of the PHA is dictated predominantly by the specific identity of the R-3-hydroxyacyl-CoA that is provided by the metabolic pathway in the organism (for example, in the above scheme, the PHA would be poly-(3-hydroxybutyrate)). PHA synthases are known to utilize R-3-hydroxyacyl CoA intermediates as substrates, but have different substrate specificities with respect to carbon chain length of the R-3-hydroxyacyl CoA.

The fatty acid biosynthetic pathway is capable of producing R-3-hydroxyacyl-*ACP* molecules ("ACP" is acyl carrier protein) as intermediates. However, the enzyme PHA synthase (*phaC*) cannot use R-3-hydroxyacyl-ACP as a substrate to make PHA. PHA synthase can only utilise R-hydroxyacyl-*CoA* ("CoA" is Coenzyme A) as a substrate.

Thus, the ACP intermediates of the fatty acid biosynthesis pathway could be made available for medium chain length PHA production, *if* the pathway could be engineered to include one or more metabolic steps that swap ACP for CoA on medium chain length R-3-hydroxyacyl-ACP molecules. The ACP intermediates must be converted to the CoA form in order to be acceptable substrates for PHA synthases.

The present application discloses a new way to produce medium chain length PHAs in organisms that do not naturally produce them, by tapping into the fatty acid biosynthetic route to obtain R-3-hydroxyacyl-CoA substrates for incorporation into PHA polymers. In other words, the specification discloses a new system and method of providing R-3-hydroxyacyl-CoA substrate that is independent of the naturally occurring *phaA*- and *phaB*-encoded metabolic pathway, and which leads to the production of medium chain length PHAs.

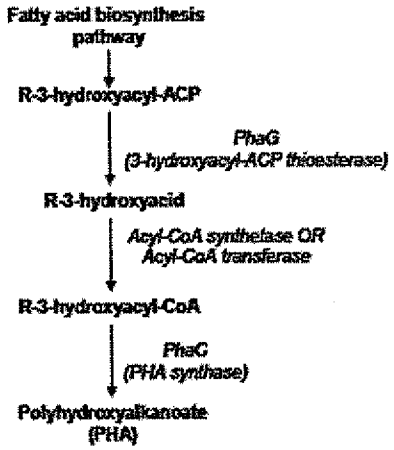
As disclosed in the specification, this was accomplished by using organisms selected and engineered to express:

(i) an enzyme with 3-hydroxyacyl-ACP thioesterase activity, such as that encoded by the *PhaG* gene, to convert R-3-hydroxyacyl-ACP into free R-3-hydroxyacid, and

(ii) an enzyme that has either an acyl-CoA synthetase OR an acyl-CoA transferase, either of which has activity to convert free R-3-hydroxyacid to R-3-hydroxyacyl-CoA.

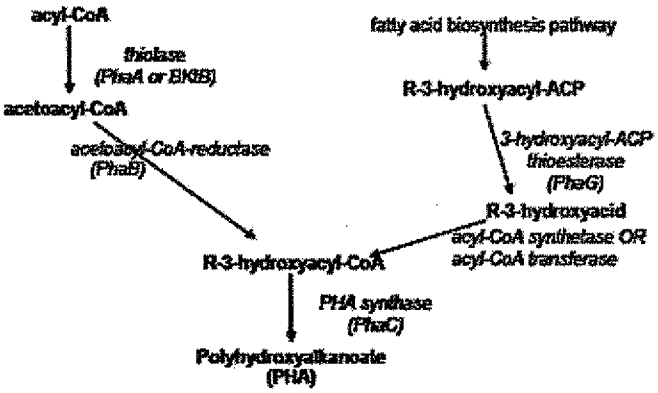
The R-3-hydroxyacyl-CoA can then be used as a substrate by a PHA synthase enzyme to produce PHA polymer. This can be represented in the following diagram as:

(Diagram B)



In effect, the pathway disclosed in the specification (shown below on right) intersects with the naturally-occurring *R. eutropha* pathway (shown on left):

(Diagram C)



As shown above, these two pathways use different initial substrates, and different pathways, to make PHA. Accordingly, the organisms must express different enzymes to utilize the pathway on the right, than are required for the pathway on the left. An organism that utilizes the pathway on the left would not inherently also be capable of possessing or being able to utilize the pathway on the right.

The enzyme *PhaG* was previously suggested to be useful in linking fatty acid biosynthesis to PHA production (see specification, page 2, line 24 to age 3, line 19), but only on the basis that it acted as an acyl-ACP CoA transferase. In other words, the prior art (including the Kruger reference cited by the examiner) suggested that *PhaG* was capable of directly converting R-3-hydroxyacyl-ACP into R-3-hydroxyacyl-CoA in a single step. However, as discussed at page 3, lines 8-14 of the present application, previous attempts to produce medium chain length PHAs by transforming *E. coli* with a gene encoding acyl-ACP-CoA transferase and medium chain length PHA synthetase were unsuccessful.

As disclosed in the present specification at page 3, line 27 to page 4, line 1, *PhaG* was found to found to function as a thioesterase rather than as an acyl-ACP-CoA transferase and so was not directly providing the 3-hydroxyacyl-CoA substrates required by the PHA synthase.

In other words, applicants have shown that the *PhaG* enzyme alone is not capable of providing R-3-hydroxyacyl-CoA for the production of PHA from the fatty acid biosynthesis intermediate R-3-hydroxyacyl-ACP. Rather, an additional enzymatic step is required, that is, the provision of an acyl-CoA synthetase or acyl-CoA transferase enzyme that converts free R-3-hydroxyacid into R-3-hydroxyacyl-CoA.

The present specification discloses that the successful production of medium chain length PHAs from intermediates of the fatty acid biosynthetic pathway can be achieved by transforming organisms to express an enzyme having 3-hydroxyacyl-ACP thioesterase activity (e.g., *PhaG*) and one or more transgenes encoding enzymes having the catalytic activity of acyl-CoA synthetase or acyl CoA transferase, so that medium chain length PHA accumulates through the fatty acid biosynthesis pathway. *PhaG* alone cannot produce PHA from R-3-hydroxyacyl-ACP.

None of the cited art recognized the need to provide *PhaG* in combination with an acyl-CoA synthetase or acyl CoA transferase in order to produce PHAs, because there was no previous recognition that *PhaG* functions as a thioesterase.

(b) Rejection of claims 1, 3-9, 11 and 13-26 under 35 U.S.C. §102(a)

Claims 1, 3-9, 11 and 13-26 were **newly** rejected under 35 U.S.C. § 102(a) as anticipated by Poirier *et al. Plant Physiology* 121:1359-1366, 1999; "Poirier".

Poirier discloses the production of medium chain-length polyhydroxyalkanoates from intermediates of beta-oxidation of fatty acids, in order to study fatty acid degradation in developing seeds of *Arabidopsis* (Abstract). The authors created several constructs containing the *PhaC1* gene (which encodes a PHA synthase enzyme), under the control of several different promoters. One construct was also made which included the PHA synthase gene and the *FatB3* gene, which encodes a medium chain length acyl-ACP-thioesterase.

The Poirier reference produces medium chain-length polyhydroxyalkanoates from intermediates from **fatty acid beta-oxidation.** Fatty acid beta-oxidation degrades fatty acids.

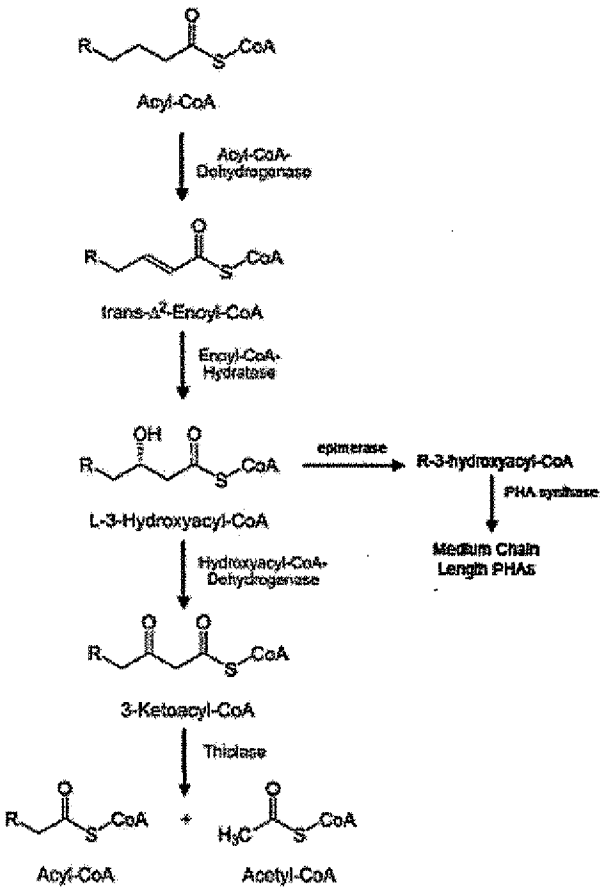
The present application produces medium chain-length polyhydroxyalkanoates from intermediates from **fatty acid biosynthesis.** Fatty acid biosynthesis builds and elongates fatty acid chains.

The two are very different pathways, and are not mirror-image reversals of each other. They involve different enzymes and steps.

The beta-oxidation fatty acid degradation cycle acts on long-chain fatty acids, and through four steps, removes two carbons in the form of an acetyl-CoA. The fatty acid, now two carbons shorter, goes through the cycle again, which produces another two-carbon acetyl-CoA and a fatty acid chain reduced by those two carbons. The cycle is repeated until the fatty acid is completely degraded to acetyl-CoA. This is shown in Diagram D below.

During the cycle, S-3-hydroxyacyl-CoA is produced as an intermediate. This molecule can be diverted to make medium chain-length polyhydroxyalkanoate *if* an epimerase is provided to convert the S-3-hydroxyacyl-CoA to R-3-hydroxyacyl-CoA. PHA synthase can only act upon R-3-hydroxyacyl-CoA.

(Diagram D)



The claimed subject matter does not involve the beta-oxidation pathway. Instead, it uses a product of the fatty acid biosynthesis pathway as its raw material.

In contrast to the beta-oxidation pathway, fatty acid biosynthesis builds and elongates fatty acid chains. During this elongation cycle, R-3-hydroxyacyl-ACP is made. PHA synthase cannot act on this molecule, and so the R-3-hydroxyacyl-*ACP* must be converted to R-3-hydroxyacyl-*CoA*. This is accomplished by a two-step process (see Diagram B, above). First, a thioesterase, *e.g.*, R-3-hydroxyacyl-ACP thioesterase, converts the R-3-hydroxyacyl-ACP to an R-3 hydroxyacid. This molecule must then be converted to R-3-hydroxyacyl-CoA by either a

synthetase (*e.g.*, acyl-CoA-synthetase) or a transferase (*e.g.*, acyl-CoA-transferase). Once the conversion to R-3-hydroxyacyl-CoA has occurred, a PHA synthase can act on this substrate, and convert it to PHA.

Clams 3 and 4- 6 are novel over Poirier

In addition to the argument above with respect to claim 1, Poirier does not disclose providing an organism with a transgene encoding any acyl-CoA synthetase, even less so a 3-hydroxyacyl-CoA synthetase or the *alk* gene. Therefore, claims 3 and 4-6 are novel over Poirier.

Claims 13 and 15 are novel over Poirier

Poirier does not disclose providing a construct comprising a transgene encoding the catalytic activity of acyl CoA-synthetase or acyl CoA transferase as required by the claims. Therefore, claims 13 and 15 are novel over Poirier.

Claims 16-18 are novel over Poirier

Claims 16-18 depend from claim 13 and additionally require the construct further comprise a transgene encoding a PHA synthase. Poirier does not disclose a method of providing an organism with gene constructs having three enzyme activities. Therefore, claims 16-18 are novel over Poirier.

Claims 20 and 23-25 are novel over Poirier

Poirier does not disclose a method of making medium chain length PHA as claimed, that requires growing an organism producing PHA and expressing a transgene encoding an enzyme having the catalytic activity of 3-hydroxyl-ACP thioesterase and expressing one or more transgenes encoding enzymes having the catalytic activity of acyl-CoA synthetase or acyl CoA transferase. Therefore, claims 20, and 23-25 are novel over Poirier.

Claims 22 and 24 are novel over Poirier

Claims 22 and 24 depend on claim 20 and specifically define the acyl-CoA synthetase as 3-hydroxyacyl-CoA synthetase. Poirier does not disclose a method for making medium chain length PHA that involves providing any acyl-CoA synthetase, even less so a 3-hydroxyacyl-CoA synthetase. Therefore, claims 22 and 24 are novel over Poirier.

Claim 26 is novel over Poirier

Poirier does not disclose a method of making PHA in bacteria as admitted by the Examiner (page 4 of Examiner's Answer mailed on 10/18/07). Therefore, claim 26 is novel over Poirier.

(c) Rejection of Claims 1, 3-13, 15-20, 22-26, 29 and 30 as obvious under 35 U.S.C. §103(a) over Poirier in view of U.S. Patent No. 5,750,848; "Kruger", or as obvious over Kruger.

The scope and contents of the prior art

Poirier

Poirier is discussed above.

Kruger

This reference is discussed at several point in the specification (see, page 2, line 24 to page 3, line 5; page 3, lines 15-19; page 6, lines 20-24; page 8, lines 22-27; page 11, lines 1-3) as failing to show that in addition to *PhaG*, an acyl-CoA synthetase (or transferase) is also required to produce PHA from fatty acid biosynthesis intermediates. Kruger discloses a portion of the metabolic pathway, but unbeknownst to the authors of this reference, their version of the pathway contained a gap.

Kruger discloses a genomic fragment harboring the *PhaG* gene, cloned by complementation of *Pseudomonas putida* mutants defective in PHA synthesis via *de novo* fatty acid biosynthesis. According to Kruger, the *PhaG* gene is useful for the production of PHAs in bacteria and plants (Kruger, abstract).

However, Kruger does not disclose the claimed organisms or method, which require a transgene encoding an enzyme activity having the catalytic activity of 3-hydroxyacyl-ACP thioesterase **and** one or more transgenes encoding enzymes having the catalytic activity of acyl-CoA synthetase or acyl CoA transferase, such that medium chain length PHAs accumulate.

On the contrary, Kruger fails to teach or suggest that one or more transgenes encoding enzymes having the catalytic activity of acyl-CoA synthetase or acyl CoA transferase must be co-expressed with the *PhaG* gene.

The authors of the Kruger reference appear to believe that *PhaG* can convert R-3-hydroxyacyl-ACP to R-3-hydroxyacyl-CoA in one step, so that *PhaC* (the PHA synthase) can then act on the product to make PHA. As discussed above, however, applicants have found that the *PhaG* enzyme converts R-3-hydroxyacyl-ACP to R-3-hydroxyacids, which were found to accumulate in the culture medium (see specification, page 3, lines 27-30; page 8, lines 22-27). An acyl-CoA-synthetase or an acyl-CoA-transferase is then required to convert the R-3-hydroxyacid to R-3-hydroxyacyl-CoA. **This last step is not disclosed in Kruger.**

Furthermore, Kruger does not demonstrate that *PhaG* can act as a 3-hydroxyacyl-ACP thioesterase. Kruger instead proposes numerous possible activities for the isolated *PhaG*:

- Fig. 1 proposes that *PhaG* could be functioning as a ligase, a thioesterase, or as an acyltransferase on four different substrates;

- Col. 3, lines 16-24 suggests, at best, a CoA-ACP acyltransferase activity for *PhaG*, which directly converts acyl-ACP molecules to acyl-CoA molecules. However, the disclosure of CoA-ACP acyltransferase activity is not disclosure of 3-hydroxyacyl-ACP thioesterase activity.
- Example 10 further notes that transforming organisms with transgenes for just the *PhaG* enzyme and PHA synthase would be sufficient for the production of PHAs. This teaches the reader that the *PhaG* enzyme is capable of directly converting acyl-ACP intermediates to acyl-CoA substrates for PHA synthase. However, such an activity is not the activity of a 3-hydroxyacyl-ACP thioesterase.
- Kruger speculates on a plethora of possible other activities for *PhaG*. The reference states (see, *e.g.*, col. 5, lines 62-64 and col. 6, lines 2-8) that *PhaG* may:

(i) (i) be an R-3-hydroxyacyl CoA-ACP acyl transferase, catalyzing the conversion of R-3-hydroxyacyl-ACP to R-3-hydroxyacyl CoA;

(ii) (ii) be a CoA-ACP acyltransferase with an acyl group specificity other than the 3-hydroxy functionality;

(iii) (iii) have activity associated with a specific thioesterase (active on any of the acyl-ACP forms in Figure 1), in which case the enzyme encoded by *PhaG* additionally requires a ligase and may, as shown in Figure 1, have specificity for acyl-ACP, 3-hydroxyacyl-ACP or 3-ketoacyl-ACP;

(iv) (iv) have activity associated with a ligase, in which case *PhaG* additionally requires a thioesterase; and, *PhaG* may have specificity for the fatty acid released from acyl-ACP, 3-hydroxyacyl-ACP or 3-ketoacyl-ACP;

(v) (v) be a protein that stabilizes a catalytic protein complex which catalyzes the acyl group transfer reaction or thioesterase or ligase activity; or

(vi) (vi) be a protein that regulates or regulates a catalytic protein complex which catalyzes, the acyl group transfer reaction or thioesterase or ligase activity.

This is merely an invitation to experiment, not a disclosure of the claimed elements. One of ordinary skill in the art cannot conclude from the Kruger reference that *PhaG* encodes a 3-hydroxyacyl-ACP thioesterase, or that any other enzymes are required. When the reference is considered as a whole it is clear (particularly from col. 3, lines 16-24 and Example 10) that the Kruger authors considered *PhaG* to be a 3-hydroxy acyl-ACP-CoA transferase, not a a 3-hydroxyacyl-ACP thioesterase.

Thus, although Kruger discloses the expression of *PhaG*,

- (i) it does not recognize that *PhaG* acts as a 3-hydroxyacyl-ACP thioesterase;
- (ii) **it does not disclose or suggest that a 3-hydroxyacyl-ACP thioesterase must be co-expressed with one or more transgenes encoding enzymes having the catalytic activity of acyl-CoA synthetase or acyl CoA transferase**; and
- (iii) it does not disclose that the claimed system can be used to allow medium chain length PHAs accumulate.

Moreover, since Kruger clearly teaches that expression of *PhaG* and a PHA synthase alone are sufficient to allow production of PHA (see, *e.g.*, Example 10 of Kruger), then it actually *teaches away* from the applicant's new finding that a further transgene encoding an acyl-CoA synthetase or acyl CoA transferase should be expressed to allow medium chain length PHA production from an intermediate of the fatty acid biosynthesis pathway.

Accordingly, the claims of the present application are not obvious in view of the Kruger reference. Only Appellants have recognized that one must have three genes: the PHA synthase involved normally in PHA production, a transgene encoding 3-hydroxyacyl-ACP thioesterase,

and a transgene encoding acyl-CoA synthetase or acyl CoA transferase. Accordingly, none of the claims can be obvious over Kruger.

Claims 1, and 3-12 are not obvious over Poirier, in combination with Kruger, or over Kruger alone.

Differences between the prior art and the claims

The differences between the prior art and the claimed subject matter is discussed above.

A combination of Poirier with Kruger, or Kruger if considered alone does not recite all of the claim limitations

The Poirier reference discloses production in *Arabidopsis* of PHA from intermediates from beta-oxidation of fatty acids. This is a pathway which degrades fatty acids, and is a completely different pathway from the fatty acid synthesis pathway, which is the pathway which produces the intermediates used by the enzymes specified in applicants' claims. Both pathways can produce R-3-hydroxyacyl-CoA, which can then be converted to PHA. However, applicants' claims cover subject matter *upstream* of this particular intermediate, which is a portion of the pathway that does not involve the beta-oxidation pathway. Applicants' claims require the use of two enzymes which are not known to be involved in beta-oxidation.

As discussed above in response to the 35 U.S.C. §102(b) rejection, Poirier does not disclose the claimed organisms or methods. Kruger when considered alone, or in combination with Poirier does not teach each and every limitation of the claims as required by 35 U.S.C. §103(a).

It is clear that the Examiner is using an improper "Obvious to try" rationale in support of the obviousness rejection which invites one to try each of numerous possible choices until one possibly arrived at a successful result. For each of the enzyme activities disclosed for PhaG from

(i) to (iv) above, one would need to provide different additional enzymes or enzyme combinations in addition to PhaG, in order to arrive at PHA (*see* FIG 1 of Kruger); for example, if PhaG has the enzyme activity of (i) above, only a PHA synthase is needed. If PhaG is (ii), then for each of the three different acyl-group functionalities disclosed in FIG 1, either (a) a 2,3,-enoyl-CoA D hydratase will be needed in addition to the PHA synthase (b) a 3-ketoacyl-CoA reductase in addition to the PHA synthase or (c) β-ketothiolase (phaA) and a 3-ketoacyl-CoA reductase (phaB) in addition to the PHA synthase. If PhaG is (iii) above, then an enzymes with ligase activity is needed in addition to a phaA, phaB and PHA synthase. If PhaG is a ligase (iv), then an enzyme with thioesterase activity is needed in addition to phaA, phaB and PHA synthase. With respect to (v) above, PhaG might not even have any catalytic activity. Clearly, attributing a thioesterase activity to PhaG is nothing but an obvious to try rationale. Furthermore, the claims require a 3-hydroxyacl-ACP thioesterase, not a thioesterase as depicted in claim 1. However, a consideration of Kruger as a whole, it is clear that Kruger ascribes an (R)-3-hydroxyacyl-ACP transferase activity, since Kruger discloses that PhaG and phaC (PHA synthase) are sufficient for PHA synthesis (*see* from col. 23, line 66, until col. 24, line 1). See also, the disclosure in Kruger at least at col. 3, lines 21-25.

There is no motivation for one of ordinary skill in the art to combine Poirier and Kruger or to modify Kruger, with any expectation of success

Poirier coexpresses a plastidial acyl-ACP thioesterase and a peroxisomal PHA synthase to increase accumulation of PHA in developing seeds. Kruger, hypothesizes among eight other possibilities, that PhaG could be an acyl-ACP thioesterase; however, further discussion in Kruger would lead a skilled artisan to think that the most probable activity for this protein according to Kruger would be a CoA-ACP transferase activity (*see* Kruger, column 3, lines 20-24), since

Kruger in Example 10 states that at least the PhaG enzyme and PHA synthase would be sufficient for the production of PHAs. This implies that the phaG enzyme is capable of directly converting acyl-ACP intermediates to acyl-CoA substrates for PHA synthase. This activity (acyl-ACP-CoA transferase) has in fact been previously demonstrated (*see* the specification at least from page 2, line 24 until page 3, line 5). Therefore, there would be no motivation for one of ordinary skill in the art to combine Kruger with Poirier as the Examiner has done. Similarly, from the disclosure in Kruger and what was commonly known in the art, a skilled artisan would believe that there was **no need** for an acyl transferase to be engineered into the same organism expressing PhaG (PhaG is an acyl transferase according to Kruger), and would not be motivated to do so.

For at least the foregoing reasons, Appellants submit that claims 1, 3-12 are nonobvious over Kruger when considered alone, or in combination with Poirier.

Claims 13, 15-19 are not obvious over Kruger alone, or in combination with Poirier

Claims 13, 15-19 define a method of engineering a PHA biosynthetic pathway in a transgenic organism selected from the group consisting of bacteria and plants which produce polyhydroxyalkanoate (PHA),

the improvement comprising providing the organism with one or more constructs comprising a transgene encoding an enzyme having the catalytic activity of 3-hydroxyacyl-ACP thioesterase and one or more transgenes encoding enzymes having the catalytic activity of acyl-CoA synthetase or acyl CoA transferase so that medium chain length PHA accumulates through the fatty acid biosynthesis pathway.

For at least the reasons set forth with respect to claims 1 and 3-12 above and in response to the 35 U.S.C. §102(b) rejection, Poirier does not disclose the claimed method. Kruger does not make up for this deficiency.

For at least the reasons set forth with respect to claims 1, and 3-12 above, there would be no motivation for one of ordinary skill in the art to combine Poirier and Kruger as the Examiner has done, or modify Kruger to arrive at the claimed method. A consideration of Kruger as a whole discloses that PhaG is an acyl transferase. Thus, there would be no motivation for one of ordinary skill in the art to combine Kruger with Poirier, which specifically requires an acyl-ACP thioesterase. Similarly, with the disclosure that PhaG is an acyl transferase and directly capable of producing PHA synthase intermediates, there would no motivation for one of ordinary skill in the art to modify the disclosure in Kruger to add any transgene other than PhaG and PHA synthase, to allow accumulation of PHA in transgenic organisms.

Claims 20, 22-26, 29, and 30 are not obvious in view of Kruger

The claims define a method of making medium chain length PHA comprising growing a transgenic organism selected from the group consisting of bacteria and plants, the organism producing polyhydroxyalkanoate (PHA) and expressing a transgene encoding an enzyme having the catalytic activity of 3-hydroxyacyl-ACP thioesterase and expressing one or more transgenes encoding enzymes having the catalytic activity of acyl-CoA synthetase or acyl CoA transferase, with substrates for fatty acid biosynthesis.

For at least the reasons set forth with respect to claims 1 and 3-12 above and in response to the 35 U.S.C. §102(b) rejection, Poirier does not disclose the claimed method. Kruger does not make up for this deficiency. A skilled artisan would understand that the PHA biosynthetic genes present and isolated from bacteria that make PHAs are phaA, B and C, encoding a beta-

ketothiolase, acetoacetyl Co A reductase, and PHA synthase. Kruger in column 23, from lines 55-62 discloses that the phaG-encoding DNA can be introduced into and expressed in a variety of different eukaryotic and prokaryotic cells such as bacteria and plants to facilitate the production of PHAs therein. Kruger further discloses that optimal PHA synthesis via *de novo* fatty acid biosynthesis in bacteria and plants comprises at least two genes: phaC and phaG. A skilled artisan would not be led to conclude from Kruger, that an additional gene encoding an acyl-CoA transferase or synthetase is essential to make PHAs from medium chain substrates produced via the fatty acid biosynthetic pathway, since Kruger does not disclose or suggest the possibility that the enzyme encoded by a heterologous phaG can function both as 3-hydroxyacyl-ACP thioesterase and an acyl-ACP-CoA transferase or acyl-CoA synthetase. Therefore, Kruger does not disclose a method that leads one to the claims method of growing a transgenic organism that encodes phaG and one or more genes such as acyl-CoA synthetase or transferase to make PHAs from medium chain substrates produced via the fatty acid biosynthetic pathway.

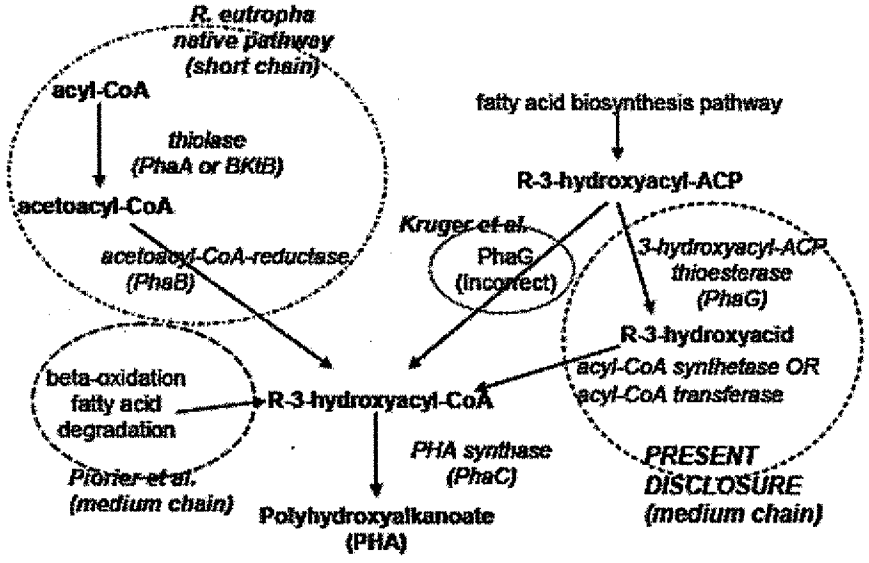
Kruger does not provide one of ordinary skill in the art with the motivation to modify the disclosure in Kruger to provide a transgene encoding an acyl-CoA synthetase or transferase, because Kruger teaches that the enzyme encoding phaG is capable of directly converting acyl-ACP intermediates into acyl-CoA intermediates, which serve as direct substrates for PHA synthases. A skilled artisan would therefore see no need to for an acyl CoA synthetase or transferase and would in fact be led away from Appellants' claimed composition and methods.

(d) Summary and Conclusions

The four metabolic pathways (the native *R. eutropha* pathway, the applicants' engineered pathway, the Kruger pathway, and the Poirier pathway) are highly complex and demonstrated by

the following diagram, showing the four pathways together, demonstrating how they each occupy "space" that is separate from the others:

(Diagram E)



From this diagram it can be seen that all four pathways are distinct from each other. All four pathways are capable of eventually making R-3-hydroxyacyl-CoA, from which polyhydroxyalkanoate can be made. However, all four reach R-3-hydroxyacyl-CoA in completely different ways. Applicants' claims to a three-enzyme transgene system for making PHA (dashed circle in lower right) is therefore distinct from that of Kruger (a (probably incorrect) two-enzyme system; small dashed oval in middle of diagram), Poirier (which uses beta-oxidation to obtain R-3-hydroxyacyl-CoA; dashed oval in lower left), and the native *R. eutropha* system (which uses a thiolase and a reductase; large dashed oval at upper left).

In summary, Kruger cannot render obvious the subject matter of applicants' claims, because this reference fails to disclose that in addition to *PhaG*, either an acyl-CoA synthetase or an acyl-CoA transferase is required to make PHA. As disclosed by applicants, providing *PhaG* is not enough. The Kruger reference neither supplies the missing elements of applicants' claims,

nor provides any suggestion or guidance as to how its teachings can be modified to provide the subject matter of the present claims.

Accordingly, the Examiner has failed to establish a *prima facie* case of obviousness and claims 1, 3-13, 15-20, 22-26, 29 and 30 are not obvious over Kruger alone or in combination with Poirier. Allowance of claims 1, 3-13, 15-20, 22-26, and 29-30 is respectfully solicited.

Respectfully submitted,

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Claims Appendix: Claims On Appeal

1. A genetically engineered organism selected from the group consisting of bacteria and plants producing polyhydroxyalkanoate (PHA),

the improvement comprising providing the organism with a transgene encoding an enzyme having the catalytic activity of 3-hydroxyacyl-ACP thioesterase and one or more transgenes encoding enzymes having the catalytic activity of acyl-CoA synthetase or acyl CoA transferase so that medium chain length PHA accumulates through the fatty acid biosynthesis pathway.

3. The organism of claim 1 wherein the acyl-CoA synthetase is 3-hydroxyacyl-CoA synthetase.

4. The organism of claim 1 comprising a transgene alkK encoding an acyl-CoA synthetase.

5. The organism of claim 1 expressing a heterologous 3-hydroxyacyl-CoA synthetase activity.

6. The organism of claim 1 expressing a heterologous 3-hydroxyacyl-CoA synthetase activity.

7. The organism of claim 1 wherein the enzyme is modified to enhance expression in the genetically engineered organism.

8. The organism of claim 1 expressing an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, medium chain length PHA synthase, and medium chain length 3-hydroxy fatty acid acyl CoA synthase, wherein the organism is a plant cell, plant tissue, or whole plant.

9. The organism of claim 8 further expressing selectable marker genes, wherein the organism is a whole plant.

10. The organism of claim 1 expressing an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, a PHA synthase that incorporates medium chain length hydroxy acids, and medium chain length 3-hydroxy fatty acid acyl CoA synthetase, wherein the organism is a bacteria.

11. The organism of claim 8 wherein expression of the transgene is targeted to a tissue or organelle selected from the group consisting of seeds, leaf, plastids, and peroxisomes.

12. The organism of claim 10 wherein the bacteria is *E. coli* and PHA accumulates within the bacteria.

13. A method of engineering a PHA biosynthetic pathway in a transgenic organism selected from the group consisting of bacteria and plants which produce polyhydroxyalkanoate (PHA),

the improvement comprising providing the organism with one or more constructs comprising a transgene encoding an enzyme having the catalytic activity of 3-hydroxyacyl-ACP thioesterase and one or more transgenes encoding enzymes having the catalytic activity of acyl-CoA synthetase or acyl CoA transferase so that medium chain length PHA accumulates through the fatty acid biosynthesis pathway.

15. The method of claim 13 wherein the construct comprises a transgene encoding a 3-hydroxy acyl-CoA synthetase.

16. The method of claim 15 wherein the construct further comprises a transgene encoding a PHA synthase.

17. The method of claim 16 wherein the organism is a plant.

18. The method of claim 16 wherein the construct expresses an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, medium chain length PHA synthase, and medium chain length 3-hydroxy fatty acid acyl CoA synthase, wherein the organism is a plant cell, plant tissue, or whole plant.

19. The method of claim 16 wherein the construct expresses an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, a PHA synthase that incorporates medium chain length hydroxy acids, and medium chain length 3-hydroxy fatty acid acyl CoA synthetase, wherein the organism is a bacteria.

20. A method of making medium chain length PHA comprising growing a transgenic organism selected from the group consisting of bacteria and plants, the organism producing polyhydroxyalkanoate (PHA) and expressing a transgene encoding an enzyme having the catalytic activity of 3-hydroxyacyl-ACP thioesterase and expressing one or more transgenes encoding enzymes having the catalytic activity of acyl-CoA synthetase or acyl CoA transferase, with substrates for fatty acid biosynthesis.

22. The method of claim 20 wherein the acyl-CoA synthetase is 3-hydroxyacyl-CoA synthetase.

23. The method of claim 20 wherein the organism further express a PHA synthase.

24. The method of claim 22 wherein the organism further express a PHA synthase.

25. The method of claim 24 wherein the organism expresses an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, medium chain length PHA synthase, and medium chain length 3-hydroxy fatty acid acyl CoA synthase, wherein the organism is a plant cell, plant tissue, or whole plant.

26. The method of claim 24 wherein the organism expresses an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, a PHA synthase that incorporates medium chain length hydroxy acids, and medium chain length 3-hydroxy fatty acid acyl CoA synthetase, wherein the organism is a bacteria.

29. The organism of claim 10 wherein the bacteria is *E. coli*, the bacteria expresses 3-hydroxyacyl-ACP thioesterase and wherein 3-hydroxy acids are secreted into the culture medium.

30. The method of claim 13, wherein the bacteria is *E. coli*, the bacteria expresses 3-hydroxyacyl-AC P thioesterase and wherein 3-hydroxy acids are secreted into the culture medium, further comprising collecting the 3-hydroxy acids from the medium.

Evidence Appendix

I. Evidence submitted with Information Disclosure Statement filed on May 8, 2002.

Madison and Huisman, *Microbiol and Mol Biol Rev.*, 63(1):21-53 (1999).

Rehm, et al., *J. Biol. Chem.*, 273(37):24044-24051 (1998)

Related Proceedings Appendix

None